IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Gladwin *et al.*
Application No. 10/563,683
Filed: October 4, 2006
Confirmation No. 3225
For: USE OF NITRITE SALTS FOR THE TREATMENT OF CARDIOVASCULAR CONDITIONS

SUBMITTED VIA EFS

Examiner: Anna Pagonakis
Art Unit: 161
Attorney Reference No. 4239-67618-07

COMMISSIONER FOR PATENTS
SUBMITTED VIA ELECTRONIC FILING SYSTEM

DECLARATION OF DR. MALTE KELM UNDER 37 C.F.R. § 1.132

I, Malte Kelm, M.D., Ph.D., declare as follows:

1. I have no financial interest in the above referenced patent application and I am not a listed inventor of the invention disclosed in the above referenced patent application.

2. A copy of my *curriculum vitae* is attached hereto as **Exhibit A**. At present, I hold a position as professor and chairman in the Department of Cardiology, Pulmonary Diseases, Vascular Medicine, and Intensive Care cardiology at the University of Düsseldorf, Germany. I have had >20 years of experience in research including work on the physiological effects of nitric oxide and inorganic nitrite and particularly the effects of these molecules on vascular tone. I have published over >200 scientific articles in scientific journals and books. By virtue of my education, training, and professional experience, I am knowledgeable about nitric oxide donors, the physiology and biology of vasodilatation, and the effects of various compounds on vasodilatation.

3. I have read Modin *et al., Acta Physiol Scand.*, 171:9-16, 2001 (attached hereto as **Exhibit B**) and familiarized myself with the teachings therein.

4. The experimental model used by Modin *et al.* is a poor model for predicting *in vivo* function because this *ex vivo* model utilizes excised rat aorta that is maintained in a modified krebs solution of neutral or acidified pH. Most, if not all, of the regulatory factors present in blood that play a physiological role in the vasodilatation process are absent from the experimental model used by Modin *et al. Of particular importance is the lack of blood in the aortic ring preparations.* Blood is expected to scavenge nitric oxide, reducing the efficacy of NO and NO donors in blood.

5. I do not believe that the Modin *et al.* reference teaches that non-acidified sodium nitrite is a vasodilator at concentrations of 25 μM or less, *in vitro* or *in vivo*. Reading Figure 2 of Modin *et al.*, I do not believe that inorganic nitrite, when applied in a neutral buffer, is an effective vasodilator of isolated segments of rat aorta at concentrations of 25 μM or less.

6. I supervised the studies published in Lauer *et al., Proc. Natl. Acad. Sci. USA*, 98, 12814-12819, 2001 (attached hereto as **Exhibit C**), a reference I co-authored. This reference teaches that no vasodilatation occurs *in vivo* at venous plasma nitrite concentrations of 130 μM (see page 12816, column 2, last paragraph) and that physiological levels of nitrite are vasodilator-inactive (see the title and abstract). My research group entitled the Lauer *et al.* paper "[p]lasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action" because of our strong belief, based on our research studies and those of others in the field, that plasma nitrite had no physiological effect on vasodilation. I believe, the results of this study were widely accepted at the time by the broad scientific community. In fact, the results published by the Gladwin group in Cosby *et al.* (Nature Medicine, 9(12):1498-1505, 2003) were very surprising to me and unexpected.

7. The Lauer *et al.* reference was published in the *Proceedings of the National Academy of Sciences of the United States of America* (PNAS), which is a scientific journal published by the National Academy of Sciences of the United States of America. The scientific community regards PNAS as a highly respected and prominent journal that publishes original scientific works. The paper was edited by a Nobel Laureate who shared in the discovery of nitric oxide.

Louis Ignarro. As such, scientific researchers in the nitric oxide field are likely read and trust the studies described in Lauer *et al.*.

8. The teachings of Lauer *et al.* are consistent with my understanding of inorganic nitrite physiology prior to October 14, 2003, which was that high concentrations of non-acidified sodium nitrite (for instance, concentrations above 200 μM) would cause vasodilation *in vitro*, but that low concentrations (including concentrations below 25 μM) were vasodilator inactive both *in vitro* and *in vivo*. Prior to October 14, 2003 I believed that inorganic nitrite was an inert oxidation product of nitric oxide metabolism. I believe that my understanding of sodium nitrite physiology prior to October 14, 2003 accurately reflects the understanding of researchers working in the field at that time.

9. Thus, prior to October 14, 2003, I would not have expected non-acidified sodium nitrite to have any beneficial therapeutic effect when administered (for instance by injection or inhalation) to a subject at circulating concentrations of 25 μM or less, regardless of the *in vitro* results in rat aorta provided by Modin *et al.*.

10. All statements made herein and of my own knowledge are true and all statements made on information are believed to be true; and further, these statements were made with the knowledge that willful false statements and like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

Date 28.08.2009

Maite Kelm, M.D., Ph.D.